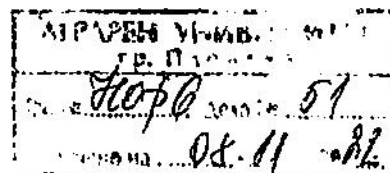


REVIEW



on the thesis for obtaining the educational and scientific degree "doctor" in: field of higher education 6. Agricultural sciences and veterinary medicine, professional direction 6.1 Plant breeding, the scientific specialty "Genetics"

Author: Todorka Angelova Srebcheva, full-time doctoral student at the Department of "Plant Physiology, Biochemistry and Genetics" at the Agricultural University, Plovdiv.

Thesis: Analysis of genes responsible for capsaicin synthesis in pepper (genus *Capsicum*)

Reviewer: Assoc. Prof. Dr. Lyubka Hristova Koleva-Valkova, Agricultural University - Plovdiv, field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological sciences, scientific specialty "Biochemistry", designated as a member of the scientific jury by order No. RD-16 -948/27.09.2022 by the Rector of AU-Plovdiv.

1. Brief introduction of the candidate.

Todorka Angelova Srebcheva was born in 1982 in the town of Kuklen. She received her secondary education at the Plovdiv Construction Technical College, majoring in Park Construction. In 2017, he obtained a bachelor's degree in "Plant Protection" at Plovdiv Agricultural University, and in 2018 - a master's degree in "Molecular Biology and Biotechnology" at Paisii Hilendarski University of Plovdiv. In the same year, he began his doctoral studies in "Genetics" at the Plovdiv Agricultural University.

Todorka Srebcheva acquired professional experience as a landscaper in the municipality of Kuklen, as a specialist in the maintenance of green areas and interior landscaping in the Berkut hotel - Brestnik, she worked in a greenhouse for potted and garden flowers and decorative tree and shrub vegetation, she was also an agronomist at the company "Agrocity" EOOD.

Since 2020, Todorka Srebcheva has been appointed as an assistant professor in Genetics in the Department of "Plant Physiology, Biochemistry, and Genetics" at the Agricultural University - Plovdiv. The teaching activity is related to performing practicals in Genetics; she participates as the head of production and educational practices and helps train diploma students in the department. Regarding scientific activity, Todorka Srebcheva participated in several national and university projects.

2. Relevance of the problem.

The topic of the dissertation is current and interesting. The pepper crop chosen for research is one of the most widely grown vegetable crops, both in Bulgaria and worldwide. The widespread distribution and cultivation of pepper is due to its nutritional value, high adaptability, productivity, as well as the content of substances useful for human health. The present study focuses on capsaicinoids, secondary metabolites

whose biosynthesis is unique to members of the *Capsicum* genus. These metabolites are widely used in the food industry, medicine, pharmacy, as a protective aerosol, as part of police equipment, and also for pest control. They are responsible for the sensation of a hot taste (spiciness) in pepper fruits. The capsaicinoid capsaicin is synthesized in the largest amount (about 69%), which, due to its high content, has become the name of all its analogs.

Analyzing the structural genes in the capsaicin biosynthetic pathway helps to elucidate the genetic basis of pungency. The degree of pungency depends on the concentration of capsaicinoids and is quantitatively inherited, while the ability of the fruit to be pungent or sweet is controlled by the dominant and recessive alleles of several genes. The identification of mutant alleles in genes with a qualitative effect on the synthesis of capsaicin and their manipulation makes it possible to screen and produce fruits with specific taste qualities - hot or sweet. Obtaining information about the genes responsible for the production of capsaicin would help to increase diversity and identify pepper forms with different content or lack of the alkaloid. In order to determine the priority of growing hot or sweet peppers, it is important to study the biodiversity of Bulgarian pepper forms and varieties. Assessment of genetic relatedness and variation is critical for effective crop plant management and improvement. All this makes the dissertation work extremely valuable and useful with scientific and applied significance.

3. Purpose, tasks, hypotheses and research methods.

The dissertation work includes a clearly and correctly formulated objective corresponding to the topic. To achieve the goal, specific and feasible tasks are specified. The literature review indicates the modern achievements in the field, but also emphasizes the problems and ambiguities in the regulation of capsaicinoid biosynthesis in Bulgarian pepper varieties. As a logical conclusion is the presence of a summary at the end with the statement of a hypothesis and setting the parameters for work. The hypothesis is well formulated and gives an idea of the doctoral student's good knowledge of the studied problem. Methods from various fields of biological and agronomic knowledge, including experimental, biochemical, genetic, selection and molecular, were used to perform the tasks. This shows the interdisciplinary nature of the research. A well-thought-out consistency is observed in the choice of analysis methods in the dissertation work, which allows comparison of the obtained results. All assays used are technically well described and allow repeatability of experiments. The data from the molecular analyzes were statistically processed with SPSS for Windows package, the data from the organoleptic analysis and the PCR results for determining the allelic status of *Pun1* were processed using the χ^2 statistic. A bioinformatic analysis of the coding regions of *Pun3*, based on information obtained from NCBI (National Center for Biotechnology Information), was also applied. A comparison of the sequences with reference genomes was also done - in the Sol Genomics Network, using BLAST (a basic search tool, finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences with sequence databases and calculates statistical significance).

All this increases the scientific value of the results and allows comparability and repeatability. The methods and experimental approaches learned by the doctoral student are a good basis for future realization in the field of scientific research.

4. Visualization and presentation of the obtained results.

The dissertation is written in a volume of 131 pages and contains 47 figures, 11 tables, and all necessary sections for the dissertation - literature review, aim and objectives, material and methods, results and discussion, conclusions, and literature. The cited literature includes 267 sources. Most of the cited sources are from the last ten years. In connection with the specificity of the topic and for the convenience of the readers, a detailed table with a list of used abbreviations is presented at the beginning. The results are presented in sections clearly, precisely, and logically, with graphs, tables, and photos. The photographic material is the author's, of very high quality, and confirms the results obtained.

5. Discussion of results and references.

The discussion of the results is in accordance with the literature review, which is detailed and well-structured in a volume of 37 pages. In it, in a logical sequence, the main supporting points necessary for developing the studied problem are arranged. The cited literature is adequately used to explain the obtained results. The discussion of the results is analytical, well-argued, and supported by up-to-date literature sources. All this reflects the in-depth knowledge of the doctoral student on the researched problem and the ability to assimilate, analyze and emphasize the most important of the currently existing knowledge.

6. Dissertation Contributions.

In the present dissertation, a large number of results were obtained, which are summarized in 8 conclusions. They are properly structured and meet the tasks set. This makes it easier to indicate the contributions of the dissertation work, which have a scientific and scientific-applied nature.

Scientific contributions

As a result of the present dissertation, 5 contributions of a scientific nature can be indicated:

- For the first time, the allelic status of the Pun1 gene was determined in the Bulgarian pepper varieties - Plovdivska Kapia, Familia, and IZK Delicacies, from the species *C. annuum* L.
- For the first time, the inheritance of the genes and their mutant alleles, responsible for the lack of pungency in F1 and F2 in the studied crosses, was traced: *C. annuum* L. – variety Plovdivska Kapia x *C. chinense* Jacq. – type Habanero; *C. annuum* L. – cultivar Familia x *C. chinense* Jacq. – Habanero type; and *C. annuum* L. – variety IZK Delicacies x *C. frutescens* L.

- The hypotheses regarding the inheritance of pungency and the lack of it in the studied peppers, validated by applying the statistical indicator χ^2 , can be used in future research by other authors.
- For the first time, the presence of additional loci with an effect on capsaicin synthesis was investigated in the F2 population of the cross *C. annuum* L. – variety Plovdivska Kapia x *C. chinense* Jacq. – type Habanero.
- Methods for conducting molecular ISSR analysis in pepper have been modified and adapted.

Scientific and applied contributions

Among the contributions of a scientific and applied nature, the following can be mentioned:

- For the first time, a successful hybridization was carried out between mother plants of the Bulgarian sweet pepper varieties Plovdivska Kapia, Familia and IZK Delicacies of the species *C. annuum* L. and hot parent plants of the species *C. chinense* Jacq. – Habanero type and *C. frutescens* L.).
- The analyzed species and varieties of pepper can be used as a potential donor in future breeding programs.
- The applied methodology for determining the allelic status of the Pun1 gene is a good strategy when testing seed lots in purity and quality programs.
- The data obtained from the sequencing and processed by the bioinformatics tools provide the basis necessary for conducting additional analyzes of the genes responsible for the synthesis of capsaicin in pepper (genus *Capsicum*).

7. Critical notes and questions.

I have no critical remarks about the thesis presented in this way, but I have the following questions:

Why do the fruits become pungent in sweet forms of pepper pollinated with pungent? What caused this?

Is there a relationship between degree of ripeness, fruit color and pungency in pungent forms of pepper?

8. Published articles and citations.

Todorka Srebcheva has presented four publications related to the dissertation work, in which the obtained results are reflected. The doctoral student is the first author of all articles. No citations of the presented publications were found at the time of the review.

- Srebcheva, T., & Bojinov, B. (2018). Genetic control of non-pungency in pepper (*Capsicum* sp.)(mini-review). *Știința Agricolă*, (2), 57-63
- Todorka, S., & Bojin, B. (2019). Identification of allelic State of the Pun-1 gene associated with the capsaicin synthesis in selected *Capsicum* (pepper) species. *Scientific works of the Union of Scientists-Plovdiv. Series C:*

Engineering and Technology, 17, 257-260.

- Srebcheva, T., & Kostova, M. (2022). Influence of the *pun1* gene on capsaicin synthesis in hybrid lines of the genus *Capsicum*. *Trakia Journal of Sciences*, 20(1), 37. DOI:10.15547/tjs.2022.01.005 Corpus ID: 248190910, ISSN 1313-3551 (online).
- Srebcheva, T., Kostova, M. (2022). Study of the inheritance of pungency in a hybrid pepper lines (genus *Capsicum*). Изследване унаследяването на лютивината в хибридна линия пипер (род *Capsicum*). *Journal of Mountain Agriculture on the Balkans (JMAB)* Journal of Mountain Agriculture on the Balkans, 2022, 25 (1), 407-422 ISSN1311-0489 (Print), ISSN 2367-836 (Online).

The presented abstract objectively reflects the structure and content of the dissertation work.

No plagiarism was found in the presented dissertation.

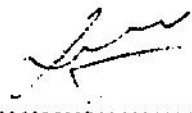
CONCLUSION:

Based on the various research methods learned and applied by the doctoral student, the correctly performed experiments, the generalizations, and the conclusions made, I believe that the presented dissertation meets the requirements of the Law on the development of the academic staff in the Republic of Bulgaria and the Regulations of the Agricultural University for its application, which gives me a reason to evaluate it **POSITIVE**.

I take the liberty of proposing to the honorable Scientific Jury to also vote positively and award Todorka Angelova Srebcheva the educational and scientific degree "Doctor" in the scientific specialty of Genetics.

Date:
Plovdiv

Reviewer:


(ASSOC. PROF. Lyubka Kideva)